10. (thrice amended) A method as in claim 9 wherein the [anti-toxins] anti-serums are anti-venoms.

11. (twice amended) A composition of matter consisting essentially of an IgG antibody made against a peptide consisting of [containing at least] five to ten amino acids from the N-terminal sequence

Leu Lys Ala Met Asp Pro Thr Pro Pro Leu Trp Ile Lys Thr Glu

in the absence of carrier protein molecule.

REMARKS

Claims 1-3 are cancelled.

Claims 5, 7, 8 and 9 are amended to obviate newly made rejections under 35 USC 112. This is applicant's first opportunity to respond to these rejections. Claim 11 is amended to better avoid prior art. The method claims are reformatted in response to a suggestion from the Examiner in a telephone conversation dated October 11, 2000, this being the only agreement reached in the telephone conversation. The amendment places the application in condition for allowance, or, by eliminating 35 USC 112 issues, in better condition for consideration on appeal. Entry is therefore requested.

The amendment to claim 5 is supported by page 4, line 16 of the specification and elsewhere. The amendment to claim 7 (roughly proportional limitation) is generally supported by Example II, pages 11-12 of the specification, the in vitro limitation is supported by page 3, lines 18-22, and the toxin on the plate limitation is supported by the Examples. The amendment to claim 8 is supported by page 8, lines 6-14 of the specification. The amendments to claim 9 are fairly supported by Example 6. Claim 9 is further amended as supported by claim 5 in view of the cancellation of claim 2, previously incorporated by reference. The amendments to claim 11 are fairly supported by page 5, lines 7-20 and page 6, lines 18-22.

Summary of the Invention

LTNF is not a toxin.

Anti-LTNF is not a toxin.

In the present invention, it has been found that anti-LTNFs will surprising react immunogenically with a vast array of toxins. Since anti-LTNF is made against all or a portion of the LTNF protein, the anti-LTNFs are reacting immunogenically against something they were not made against. This is a surprising result that could not have been predicted by one of ordinary skill. Further, it has been found that the binding affinity between anti-LTNF and toxin is roughly proportional to the lethal dose of the toxin under testing. This is a truly serendipitous discovery. Still further, the usefulness of anti-LTNF-n, anti-LTNF-15, anti-LTNF-10 and anti-LTNF-5 for this assay is shown, as well as the production of anti-LTNF-n, anti-LTNF-10, and anti-LTNF-5. Further shown is that, of the anti-LTNFs tested, anti-LTNF-10 has the highest potency, and that anti-LTNF-n, anti-LTNF-15, anti-LTNF-10, and anti-LTNF-5, while related, are not the same. Additionally, it is shown that, while anti-LTNF reacts immunogenically with toxins, (as well as with LTNF), it does not react immunogenically with anti-serums, nor with the immunogenic reaction product between toxins and their specific antiserums. In other words, once the toxin has reacted immunogenically with the anti-serum, it will no longer react immunogenically with the anti-LTNF. This is a still further serendipitous discovery, in that it enables free toxin in a partially neutralized mixture of toxin+antiserum to be assayed, and the neutralizing potency of conventional anti-serums determined, without the killing of animals.

Rejection under 35 USC 102

Claims 1-3, 11-13 and 14-15 stand finally rejected as anticipated over Lipps et al., US 5,576,297, November 19, 1996. This rejection is traversed, but is obviated with respect to claims 1-3 by their cancellation and submitted as obviated with respect to claims 11-13 by amendment to claim 11.

In view of the cancellation of claims 1-3, reconsideration of the rejection as based on these claims is requested.

Claims 11-13 as amended distinguish the disclosure of Lipps by the recitation of "consisting essentially of an IgG antibody made against a peptide consisting of five to ten amino acids" of the recited sequence. The portion of Lipps upon which the rejection is based states:

"...the synthetic LTNF is immunogenic, since mice immunized with it were able to produce specific antibodies, which reacted with both natural and synthetic LTNF..."

The antibodies disclosed by Lipps are made against synthetic LTNF, which in the present terminology is anti-LTNF-15. Since claim 11 is now directed toward antibodies made against synthetic LTNF five to ten, claims 11-13 distinguish. Reconsideration and withdrawal of the 35 USC 102 rejection of these claims is requested.

Claims 14 and 15 distinguish Lipps on the basis that the process of contacting the toxin with the recited antibody is conducted in vitro. In response to the previous office action, a request was made that the Examiner point to the portion of the reference where Lipps is said to teach "that his antibodies react with both natural and synthetic toxins". This was not done. Lipps teaches that anti-LTNF-15 reacts with both LTNF-n and LTNF-15. LTNF-n and LTNF-15 are toxin neutralizers, the nemesis of toxins. The claim points toward reacting the anti-LTNF with the toxin, rather than with the nemesis. That the biological toxin will react immunologically with the

antibody to LTNF is extraordinary.

Reconsideration of the 35 USC 102 rejection of claims 14-15 is requested.

Rejection under 35 USC 103

Claims 1-17 stand rejected as unpatentable over Lipps et al., in view of Harlow and Lane, 1988 Antibodies: A Laboratory Manual; Chapter 7 and 14. This rejection is traversed.

It is asserted in the Office Action that it would be obvious to "use the teachings of Lipps et al to raise monoclinal and polyclonal antibodies to LTNF and use it as a reagent for immunoassays such as ELISA."

The reasoning is not applicable to the method claims, none of which cover analyzing for LTNF. It is the teaching of applicant's present application that anti-LTNF will also recognize toxins that provides the motivation for raising antibodies to LTNF and using them for immunoassays involving toxins, or for assessing the neutralizing potency of anti-serums.

It is very surprising that anti-LTNF will reacts immunologically with toxins, as well as with LTNF. Normally, an antibody would be expected to react only with the antigen against which it was produced. While LTNF was a universal toxin neutralizing factor, enabling toxins to be neutralized, anti-LTNF is a universal antibody for toxins, enabling toxins to be detected, and, it turns out, also enabling them to be assessed.

Regarding the amendment to claim 11, pages 72-76 of Harlow and Lane are attached. Page 74, second paragraph, states:

> "Because of their size, peptides may not be immunogenic on their own. To elicit an antibody response directly, they must contain all of the features of any immunogen, notably they must have an epitope for B-cell binding and a site for class II-T-cell receptor

binding. Some peptides, even surprisingly small ones, contain both these sites (or more properly, one sequence that can serve both functions), and these peptides can be used without carriers [cites omitted]. Unfortunately, there are no methods, short of immunization, to test this, and therefore, most peptides are coupled to carrier proteins before injection."

At page 75, first paragraph, Harlow and Lane disclose

> "...preparing anti-peptide antibodies is still an empirical exercise. What works well for one immunogen may fail completely for another."

To further distinguish Harlow and Lane, claim 11 is further amended to recite that the antibody is made in the absence of carrier protein molecule. Whether or not such a composition could exist could not have been predicted by one of ordinary skill with knowledge of the references. Reconsideration is therefore requested.

Specific responses to statements made in office action

On page 3, first paragraph of the Office Action, it is stated:

"Lipps also teaches that his antibodies react with both natural and synthetic toxins, thereby anticipated claims 14-15."

Response: This is not believed to be an accurate statement of the teaching of 5,596,297. For the second time, the Examiner is requested to point out the portion of this reference which is contended to support this assertion.

On page 4, second paragraph, of the Office Action, it is stated:

"An artisan of ordinary skills would have been motivated to raise antibodies to LTNFn and LTNFs because it would have helped in using them for different assays, such as toxin or binding assay as taught by Lipps et al (5,576,297 and 5,744,449)."

Response: Lipps 5,744,449 is not prior art and its citation is without statutory basis. The Examiner is requested to point to the statutory basis for including it in the rejection. Toxin or binding assay with anti-LTNF is not taught by 5,576,297.

On page 4, last paragraph, of the Office Action, it is stated:

"...the person of ordinary skill in the art at the time the invention was made would have had a reasonable expectation of success of obtaining such antibodies and to use them in different assays for detecting toxins etc."

Response: The attached portion of Harlow and Lane makes it clear that there would be no reasonable expectation of success of the antibody recognizing anything other than what it was made against.

On page 5, first paragraph, of the Office Action, it is stated:

"Examiner understands and agrees that LTNF is a universal toxin."

Response: Lethal Toxin Neutralizing Factor is a universal antidote for toxins. It is not a toxin, nor does it react immunologically with the toxins which it counteracts.

Further on page 5, first paragraph, of the Office Action, it is stated:

"...it would have been obvious to a person of ordinary skill in the art at the time the invention was made to use teaching of Lipps et al (antibodies which are reactive to various toxins, column 8, lines 7-11) and Harlow and Lane to perform ELISA assays for anti-LTNF in vitro assay of different biological toxins."

Response: The statement "the synthetic LTNF is immunogenic, since mice immunized with it

were able to produce specific antibodies, which reacted with both natural and synthetic LTNF" does not constitute a teaching of "antibodies which are reactive to various toxins", or for that matter a teaching of an antibody which is reactive against any toxin.

Rejections under 35 USC 112

Claim 1

The rejection is obviated by the cancellation of the claim.

Claims 5, 7-8

Claims 5, 7-8 are rejected for lack of clarity in the method. The rejection is traversed, but is believed obviated by the above amendment which specifies that the process is conducted under in vitro conditions, and that the reaction is one which produces a product capable of being detected by ELISA (responsive to the objection as based on "what is the process" and "what is the immunological reaction"). As to lack of clarity in the method steps for performing the ELISA, the specification states at page 7, lines 33-34 that the ELISA can be conducted in several different ways. Nonetheless, claim 7 is amended to recite that the anti-LTNF is in a fluid state and the toxin is attached to a plate, which is the technique used in the Examples, and the rejection should be obviated at least as to it. Further, claim 7 as amended recites: "conducting an ELISA binding or ELISA titer on the product of the immunological reaction and obtaining a numerical result which is roughly proportional to the toxicity of the at least one biological toxin. as determined by animal bioassay" which should obviate the rejection as based on lack specificity as to how the product is identified by ELISA and how the ELISA and bioassay are compared. In this regard, claim 8 is further amended to recite that the ELISA is carried out by antigen capture format employing a second antibody in a further effort to obviate the rejection at least as to it. Reconsideration is therefore requested.

Claim 9

Claim 9 is rejected as being vague and indefinite for not having clear steps for assessing neutralizing potency. Claim 9 is amended to specify assessment of antiserum and is amended to recite more clear method steps regarding the provision of the neutralizing index, and the requirement of normal serum is set forth. Reconsideration is requested.

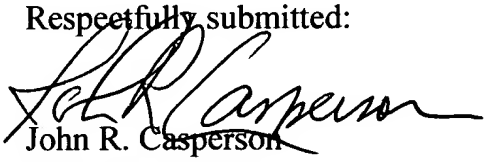
Claims 5 and 14.

Claims 5 and 14 are rejected as being duplicative. Claim 5 is narrower in its recitation of the toxin and further requires ELISA detection. What would be an infringement of one claim is not necessarily an infringement of the other. The claims are thus of varying scope and are not duplicative. Reconsideration is requested.

Conclusion

In view of the forgoing amendment and remarks, reconsideration and withdrawal of all grounds of rejection and early notice of allowance is respectfully solicited.

Respectfully submitted:



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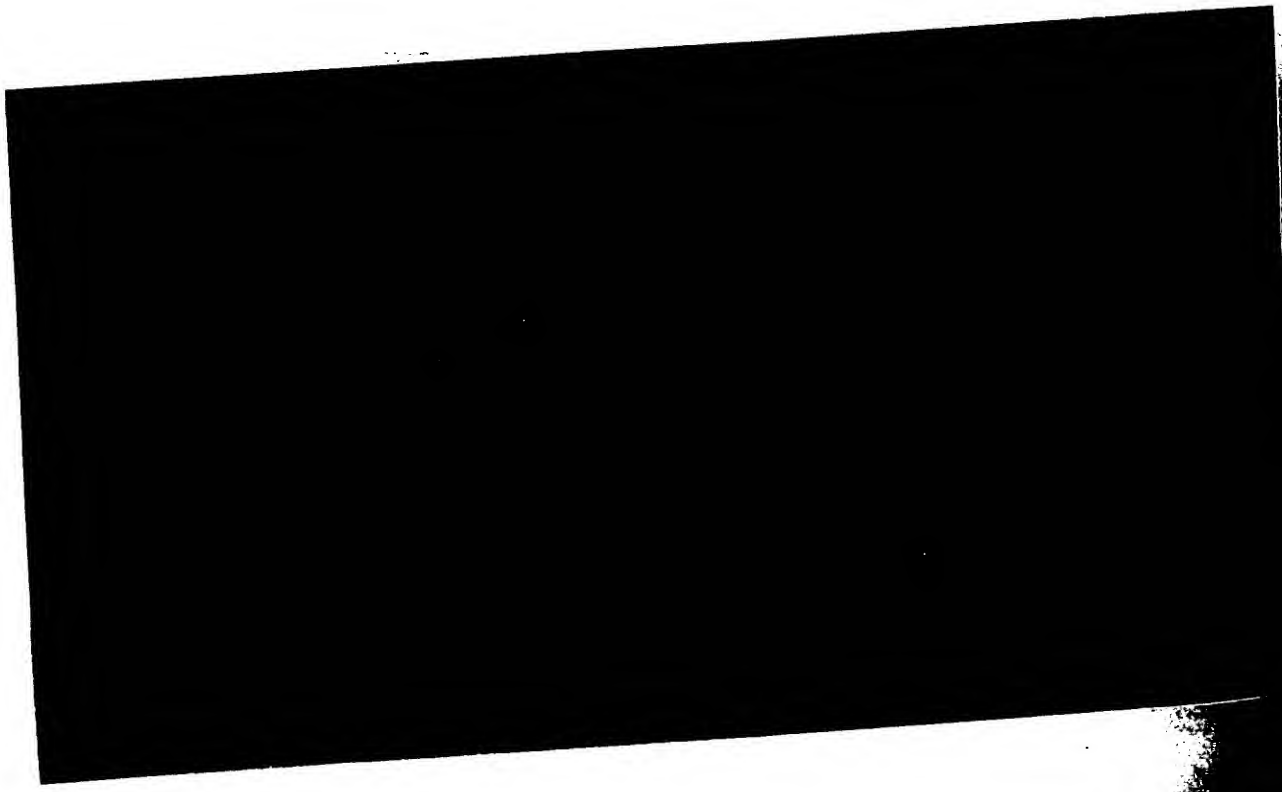
■ Haptens

Many small chemicals can be used to raise antibodies, if they are coupled to larger protein molecules. The small compounds are known as haptens, while the proteins to which they are coupled to are called carriers. The haptens themselves serve as epitopes for binding to the antibodies on the B-cell surface, and the carriers provide the class II–T-cell receptor binding sites. In general, haptens should be coupled to soluble carriers such as bovine serum albumin (BSA) or keyhole limpet hemacyanin (KLH). The coupling mechanism will vary with each hapten, but many of the bifunctional coupling reagents listed in Table 5.5 (p. 130) will be helpful. Also, the techniques on the coupling of synthetic peptides to carriers on p. 78 may be applied. In general, approximately 1 mole of hapten per 50 amino acids of carrier is a reasonable coupling ratio.

■ Synthetic Peptides

The use of synthetic peptides as immunogens has been an important technique in the elucidation of the properties of an antibody response (e.g., Goebel 1938; Anderer 1963; Anderer and Schlumberger 1965; Sela 1966, 1969; Arnon et al. 1971). Recently, as more DNA sequences



and their corresponding protein sequences have become known, synthetic peptides have been used to prepare antibodies specific for previously uncharacterized proteins (Sutcliffe et al. 1980; Walter et al. 1980; and reviewed in Lerner 1982, 1984; Walter 1986; Doolittle 1976; and in Ciba Foundation 1986). Peptides are normally synthesized using the solid-phase techniques pioneered by Merrifield (1963). The synthetic peptides are purified and coupled to carrier proteins, and these conjugates are then used to immunize animals. In these cases, the peptides serve as haptens with the carrier proteins, providing good sites for class II–T-cell receptor binding. Peptide–carrier conjugates seldom fail to elicit a response because of tolerance. Consequently, the peptides can usually be seen as epitopes, and high-titered antisera commonly are prepared. Characteristically, these antibodies will bind well to denatured proteins, but may or may not recognize the native protein.

The two most important advantages of anti-peptide antibodies are that they can be prepared immediately after determining the amino acid sequence of a protein (either from protein sequencing or from DNA sequencing) and that particular regions of a protein can be targeted specifically for antibody production. Rapid conversion from DNA sequence information to antibodies has enormous potential for application in molecular biology. Similarly, the production of site-specific antibodies has immediate implications for functional and clinical studies.

The major problem that is encountered when preparing anti-peptide antibodies is whether they will recognize the native protein. Assays that need or benefit from anti-native antibodies, such as immunoprecipitation, many cell staining techniques, or immunoaffinity purification, will succeed only when the peptide sequence is displayed on the surface of the native molecule in a conformation similar to the peptide–carrier conjugate. Therefore, the successful production of anti-peptide antibodies is often determined by the researcher's ability to predict the location of certain peptide sequences in the three-dimensional structure of the protein.

Because of their size, peptides may not be immunogenic on their own. To elicit an antibody response directly, they must contain all of the features of any immunogen, notably they must have an epitope for B-cell binding and a site for class II–T-cell receptor binding. Some peptides, even surprisingly small ones, contain both these sites (or more properly, one sequence that can serve both functions), and these peptides can be used without carriers (e.g., see Beachy et al. 1981; Lerner et al. 1981; Dreesman 1982; Jackson 1982; Atassi and Webster 1983; Young et al. 1983). Unfortunately, there are no methods, short of immunization, to test this, and therefore, most peptides are coupled to carrier proteins before injection. An exciting recent development is the use of synthetic class II–T-cell receptor sites synthesized directly with the desired epitope (Francis et al. 1987; see also, Good et al. 1987; Borras-Cuesta et al. 1987; Leclerc et al. 1987). Although there are not enough cases to determine how widely applicable this approach will be, the concept is provocative. With this strategy, the peptide of interest is synthesized as either an amino- or carboxy-terminal extension of a known class II–T-cell receptor site. The synthetic peptide, now containing both sites, is injected without coupling and used to induce an antibody response. The first experiments using this approach look very promising, and this may become an important alternative to coupling with carrier proteins.

Peptides usually are synthesized with an automated machine using solid-phase techniques. The methods for synthesis and purification of the peptide are beyond the scope of this book. However, to judge the success of the coupling reaction and to determine the number of moles of peptide bound to the carrier, a small proportion of the peptide needs to be labeled. This can be done by including a small amount of ^{14}C-labeled amino acid in the synthesis or by iodinating a sample of the peptides on a tyrosine or histidine residue (see p. 324) after the synthesis. A small sample of these iodinated peptides can then be added to the coupling reactions to ascertain the success of the coupling.

During immunization, antibodies to the carrier proteins or the coupling agent will also be produced, and these are normally removed by affinity-purifying the anti-peptide antibodies on a column prepared with conjugates of the peptide and a second carrier molecule. Techniques for affinity purification of the antibodies are described in general on p. 313.

Probably the most frequently asked question concerning synthetic peptides is what sequence should be used for the immunogen (reviewed in Doolittle 1986). Although there is no one correct answer, enough anti-peptide antibodies have been raised to make suggestions for peptide choices. However, preparing anti-peptide antibodies is still an empirical exercise. What works well for one immunogen may fail completely for another.

Choosing the Appropriate Peptide Sequence

With careful synthesis, coupling, and immunizations, most sequences can be used to induce antibodies specific for the peptide itself. When considering which sequence to use, most people actually want to know how likely will it be that the anti-peptide antibodies will recognize the native protein. Early work suggested that peptides containing hydrophilic amino acids (Hopp and Woods 1981, 1983; Kyte and Doolittle 1982) and proline residues were more likely to be exposed on the surface of the native protein than other sequences, and many peptides have been prepared using these criteria. In assessing the value of these criteria, hydrophilicity is required but is not sufficient to predict the surface location of a particular sequence. Many strongly hydrophilic amino acid sequences are buried in water pockets or form inter- or intramolecular bonds and are thus excluded from interactions with anti-native antibodies. Therefore, hydrophilicity can be thought of as required but not sufficient for choosing peptide sequences (see p. 661 for hydrophilicity values). Hydrophilic peptides are also more likely to be soluble for coupling reactions.

The presence of proline residues in synthetic peptides originally was suggested because β-turns often form portions of known epitopes. However, the presence of proline residues in peptides does not have much predictive value when antisera are tested for binding to the surface of native proteins. Although many excellent anti-peptide antisera have been prepared against sequences with proline residues, there is not sufficient evidence to target prolines when designing peptides.

More recently, several workers have noted that carboxy-terminal sequences often are exposed and can be targeted for anti-peptide sequences. Although using carboxy-terminal sequences does not guarantee that the resulting antibodies will recognize the native protein, a surprisingly high percentage will. Similarly, many amino-terminal regions are exposed, and these also may make good targets.

Another potentially useful parameter for selecting peptide sequences is the "mobility" of the amino acid residues. Originally, it was noted that the regions of a protein that become epitopes often have a higher temperature than other regions, as determined by NMR and X-ray structure (Moore and Williams 1980; Robinson et al. 1983; Tainer et al. 1984; Westhof et al. 1984). Higher temperature in crystallography and NMR distinguishes regions that are more mobile from

regions that are more static. These observations have led to the suggestion that stretches of amino acids that are more flexible are more likely to be epitopes. In the preparation of anti-peptide antibodies, when a peptide is coupled to a carrier molecule, it has a different local environment than in the original protein. When choosing a sequence for antibody production, a region of the protein that is more flexible will be more likely over time to form a structure that is similar to the peptide–carrier conjugate. Although the measure of mobility may become a useful criterion for selecting good peptide sequences, it has not been tested in enough detail to determine whether it will have any predictive value.

At present, a reasonable order of suggestions for choosing peptide sequences would be:

1. If possible, use more than one peptide.
2. Use the carboxyl-terminal sequence if it is hydrophilic and if a suitable coupling group is available or can be added.
3. Use the amino-terminal sequence if it is hydrophilic and if a suitable coupling group is available or can be added.
4. Use internal hydrophilic regions; perhaps using longer peptides.

Size of the Peptide

The smallest synthetic peptides that will consistently elicit antibodies that bind to the original protein are 6 residues in length. Responses to smaller peptides are typically weak or will not recognize the protein of interest, either in a native or denatured state. Since epitopes consisting of smaller regions have been reported, the lower limit presumably reflects the difficulty of recognizing the smaller peptides coupled to carriers. With peptides of 6 amino acids or slightly larger, the responses vary. Some will generate good antibodies and some will not. Generally, peptides of approximately 10 residues should be used as a lower limit for coupling.

In the literature two strategies are suggested for peptide length. One school suggests using long peptides (up to 40 amino acids long) to increase the number of possible epitopes, while other authors argue that smaller peptides are adequate and their use ensures that the site-specific character of anti-peptide antibodies is retained. Both strategies have been used successfully. Two important preliminary questions to consider are: (1) Does the anti-peptide serum need to recognize the native protein? If so, use longer peptides or prepare anti-peptide antisera against multiple peptides. (2) How good is your peptide synthesis facility? Peptides over 20 residues in length are increasingly difficult to synthesize, yielding products with inappropriate side reactions. Longer peptides also are more likely to contain residues that make the coupling to carrier molecules more difficult. The correct decison between peptides with 10–15 residues and longer peptides will depend on the experimental design and will normally be a compromise between these factors. The safest choice, but also the most expensive, will be to prepare multiple small peptides of 10–15 amino acids in length from various regions of the sequence.